PRIMER NOTE

Sequence variation of intergenic mitochondrial DNA spacers (mtDNA-IGS) of *Phytophthora infestans* (Oomycetes) and related species

R. A. M. WATTIER,*L. L. GATHERCOLE,*S. J. ASSINDER,*C. J. GLIDDON,*K. L. DEAHL,†D. S. SHAW* and D. I. MILLS‡

*School of Biological Sciences, University of Wales, Bangor, Gwynedd, LL57 2UW, UK, †Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331–2902, USA, ‡Vegetable Laboratory, ARS, USDA, Beltsville, MD, USA

Abstract

The potato late-blight disease is caused by the pseudofungus *Phytophthora infestans* (Oomycetes). This pathogen was of historical importance as it caused the Irish Potato Famine. There is currently a worldwide resurgence of the disease. Following worldwide migrations as well as being able to discriminate *P. infestans* from related species are key issues. We present sequence variation of five inter-genic mitochondrial DNA spacers (mtDNA-IGS) for *P. infestans* and four related taxa. Intra and inter-taxon variation was observed showing potential for both molecular ecology and molecular systematic.

Keywords: genetic marker, mtDNA intergenic spacers (mtDNA-IGS), *Phytophthora infestans*, potato late-blight disease

Received 22 July 2002; revision accepted 20 November 2002

The filamentous pseudofungus *Phytophthora infestans* (Oomycetes) causes late-blight disease of potato and was responsible for the Irish famine in the 1840s. The pathogen remains highly destructive, and the last 25 years have seen a worldwide resurgence in the disease which has been associated with migration(s) of the pathogen from Mexico, its centre of diversity (Fry and Goodwin 1997). In addition, the taxonomic status of many isolates from South America remains obscure (Ordonez *et al.* 2000). In Mexico, two related taxa, *P. mirabilis* (Goodwin *et al.* 1999) and *P. ipomoeae* (Flier 2001) were only very recently recognised as distinct species.

The aim of this study was to ascertain whether noncoding intergenic mitochondrial (mt)DNA spacers (mtDNA-IGS) showed sequence variation that could (i) provide additional polymorphism within *P. infestans* to aid in population genetics and phylogeography, because so far only four mitochondrial haplotypes have been described worldwide (e.g. Griffith & Shaw 1998), and (ii) be used for molecular identification and phylogeny of species closely related to *P. infestans*.

Four primer pairs (D88, F149, H139 and P191, Table 1) were designed, and one primer pair (ATP9) was previously described in *P. cinnamomi* (Dobowolski *et al.* 1998;

Correspondence: R. A. M. Wattier. Fax: (44) 01248 370731, E-mail: Remi wattier@hotmail.com

Table 1) but had never been used for sequences analysis. Primers were designed from adjacent coding sequences to amplify five mtDNA-IGS and are based on the complete mtDNA genome sequence (NCBI NC_002387) of *P. infestans*.

Of 23 isolates selected for analysis (Table 2), 15 of *P. infestans* were from UK, USA, Canada, Mexico and Ecuador, including all four mtDNA haplotypes (Ia, Ib, IIa and IIb) already described. Two isolates from *Solanum brevifolium* in Ecuador were of ambiguous taxonomic status, possibly *P. infestans* (Ordonez *et al.* 2000). Two isolates each of *P. ipomoeae*, *P. mirabilis* and *P. phaseoli* were from Mexico.

Polymerase chain reactions (PCR) were carried out in a volume of 10 μ L that included approximately 5 ng template DNA, 100 μ M each dNTP, 200 nM each primer, 10 mM Tris-HCl, 50 mM KCl, 0.2 mg/mL gelatin and 0.5 Unit Taq DNA polymerase (all chemicals from Invotrogen). The concentration of MgCl₂ varied as described in Table 1. DNA was amplified in a Mastercycle Eppendorf thermocycler beginning with an initial denaturation at 94 °C for 2 min, followed by 35 cycles consisting of 0.5 min denaturation at 94 °C, 0.5 min annealing at a temperature indicated in Table 1, 1 min of elongation at 72 °C, and a final extension step at 72 °C for 3 min. PCR products were monitored by electrophoresis in 2.5% agarose gels. The PCR products were purified using the exonuclease and shrimp alkaline

Table 1 PCR primers for amplification of mtDNA intergenic sequences (mtDNA-IGS) in Phytophthora infestans

Name*	Genest	Position‡	Primers (5′–3′)	$T_{\rm a}$ (°C)	MgCl ₂ (mM)	PCR (bp)
D88	orf32	8552	GGAAATTAAAAAAGCCAACGC	50	2.0	130
	cox1	8638	AAAAGCCATCTTGTTGACCAT			
F149	cox1	10118	TATATGTAACATTAGTTCACGG	50	2.0	235
	atp9	10266	AAGTAGCTAATCCTGCACC			
ATP9§	atp9	10494	TTTATTCTGTTTAATGATGGC	55	1.5	382
	nad9	10655	CAGCACAAATTTCAGATAATAC			
H139	cob	12433	GAAGCATATTTAGTACGTTATAG	55	1.5	226
	atp1	12572	AAAATTAAATACTTTAGCTATCG			
P191	ymf16	30379	TGCTTTAATTACTCCACCAGA	50	2.0	335
	trnC	30570	CATTACCATTATGTTATCTAGC			

^{*}Name for the mtDNA-IGS.

Table 2 Distribution of nucleotide polymorphism in five mtDNA intergenic spacer sequences of Phytophthora infestans and related species

	GS*	D88	F149	ATP9	H139	P191
Site†			1111111111111	111111111111	111111111111111111111111111111111111111	333
		88888	000000000000	00000000000	2222222222222222222222222222222	000
		56666	11111111111111	55555555557	444444444444444555555555555555555	334
		81223	1333458888999	122344455687	44444455666778890122244667777777	571
		99014	7348074689013	878778956923	234567690154979884789008901234567	550
Isolates‡/Ref§		TTTTC	AGACACTCACGAC	ATTTCTTCGGTT	TATTTCTTTATGACATTAATAA-TTTTTTATTT	GTC
Pi-ORE2	Ia					
Pi-UK2, Pi-MEX2,	Ia			·AA		
Pi-ORE1, Pi-MEX1	Ia			·AA	·····AA··A····	
Pi-UK1, Pi-ORE3	Ιb					
Pi-UK3, Pi-EC1, Pi-EC2, Pi-EC3	IIa	• • •	$\cdots _{T}\cdots \cdots \cdots$			
Pi-UK4	IIb	• • •	$\cdots _{T}\cdots \cdots \cdots$			
Pi-ORE4, Pi-ORE5	Ilb	• • •	$\cdots _{T}\cdots \cdots \cdots$	·AA		
Pi-CAN1	IIb	• • •	$\cdots _{T}\cdots \cdots \cdots$	·AA	· · · · · · · · · · · · · · · · · · ·	
Pi?-ECbr1, Pi?-ECbr2	_	••	$-\cdots$ G·CTGTAGA	····AC····	·····AAA··AT···A····	A٠٠
Pipom-1	_	C•	$T \cdot \cdot \cdot G \cdot \cdot A \cdot \cdot A \cdot \cdot$	·AAC	$\cdots \cdot \texttt{A} \cdot \cdots \cdot \texttt{T} \cdot \cdot \texttt{GA} \cdot \texttt{G} \cdot \cdots \cdot \texttt{A} \cdot \cdots - \cdots$	A٠٠
Pipom-2	_	C•	$T \cdot \cdot \cdot G \cdot \cdot A \cdot \cdot A \cdot \cdot$	·AAC	$\cdots \cdot \texttt{A} \cdot \cdots \cdot \texttt{T} \cdot \texttt{TGA} \cdot \texttt{G} \cdot \cdots \cdot \texttt{A} \cdot \cdots - \cdots$	A٠٠
Pmira-1	_	• • •	··G·G····	·····CC·A···	·····AAAG·AT···A··	A٠٠
Pmira-2	_	• • •	··G·G·····	•AA•T•C•A•••	$\cdots \cdot \texttt{A} \cdots \texttt{C} \cdot \texttt{T} \cdots \texttt{A} \cdots - \cdots \cdots \cdots$	Α··
Pphas-1, Pphas-2	_	•T	·A···T····	GAAC··CTATAC	A·····TTT·····	ACT

^{*}GS: mtDNA haplotypes as defined for P. infestans in Griffith & Shaw (1998).

§Reference sequence based on the complete genome sequence of *P. infestans* mtDNA.

phosphatase method (http://www.genetics.ucla.edu/sequencing/purification.htm). Purified PCR products were cycle-sequenced using ABI PrismTM BigDyeTM Terminator chemistry with Ampli*Taq*® DNA Polymerase, and sequenced using a Perkin-Elmer, Applied Biosystems Division, 373 A DNA Sequencer. Sequences were edited using the CHROMAS 1.51 software (Technelysium Pty Ltd, http://www.technelysium.com.au/chromas.html) and

aligned using the BIOEDIT 5.0.9. software (T. Hall, http://www.mbio.ncsu.edu/BioEdit/bioedit.html). At least two independent PCR products were sequenced for each polymorphism observed.

Polymorphic sites for the five mtDNA-IGS, D88, F149, ATP9, H139 and P191, are presented in Table 2. For the 15 *P. infestans* isolates, the observed variation was as follows. P191 was monomorphic. Polymorphisms were detected for

[†]Genes flanking the IGS sequences.

[‡]Starting and ending nucleotide position of the mtDNA-IGS (from GenBank, accession NC_002387).

[§]Primers as presented in Dobrowolski et al. (1998).

[†]Site numbers (written vertically) refer only to polymorphic sites relative to the complete genome sequence of *P. infestans* mtDNA (GenBank, Accession NC_002387); Dots indicate identity and dashes gaps.

[‡]Pi: *P. infestans*; UK: United–Kingdom; ORE: Oregon, USA; CAN: Canada; MEX: Mexico; EC: Ecuador; Pi?: isolate with ambiguous taxonomic status (Ordonez *et al.* 2000); Pipom: *P. ipomoeae*; Pmira: *P. mirabilis*; Pphas: *P. phaseoli*.

both D88 and F149 but they were in linkage disequilibrium with the polymorphisms already described (Griffith & Shaw 1998); isolates of haplotype Ia and Ib have one sequence type whereas isolates with IIa or IIb have another. Although the combination of polymorphisms detected for ATP9 and H139 did not allow subdivision of haplotypes Ib and IIa into new haplotypes, they did allow the splitting of both Ia and IIb into three new haplotypes.

The data show that the two isolates with ambiguous taxonomic status (Pi?-ECbr1 and Pi?-ECbr2) are different from the isolates of *P. infestans* from potato (Table 2). The two Pi? isolates have identical nucleotide sequences but are polymorphic for 45 nucleotides within the five targets with respect to the reference sequence (including a 10 nucleotide deletion within H139). These two isolates are also highly polymorphic with respect to the three other species examined in this study.

The high level of variation observed among species revealed the potential of these markers in taxonomy and phylogeny. Finally, intraspecific polymorphism is observed for *P. mirabilis* at ATP9 and for *P. ipomoeae* and *P. mirabilis* at H139.

Acknowledgements

The authors thank G. A. Forbes (CIP, Ecuador) who provided isolates Pi?- Ecbr1 and Pi?-ECbr2, W. G. Flier, N. J. Grunwald and L.

P. Kroon (PRI, the Netherlands) who provided isolates of *P. ipomoeae, P. mirabilis* and *P. phaseoli,* and A. Stenson (University of Wales, Bangor), N. Adair and D. Borngasser (Oregon State University) for assistance in DNA sequencing. This project was funded in part by BBSRC, the USDA Agricultural Research Service, and the OSU Agricultural Experiment Station.

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